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Cerebral cortex demyelination and oligodendrocyte precursor response to experimental autoimmune encephalomyelitis

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ABSTRACT

Experimentally induced autoimmune encephalomyelitis (EAE) in mice provides an animal model that shares many features with human demyelinating diseases such as multiple sclerosis (MS). To what extent the cerebral cortex is affected by the process of demyelination and how the corollary response of the oligodendrocyte lineage is explicated are still not completely known aspects of EAE. By performing a detailed *in situ* analysis of expression of myelin and oligodendrocyte markers we have identified areas of subpial demyelination in the cerebral cortex of animals with conventionally induced EAE conditions. On EAE-affected cerebral cortices, the distribution and relative abundance of cells of the oligodendrocyte lineage were assessed and compared with control mouse brains. The analysis demonstrated that $A2B5^+$ glial restricted progenitors (GRPs) and $NG2^+$/PDGFR-α^+ oligodendrocyte precursor cells (OPCs) were increased in number during "early" disease, 20 days post MOG immunization, whereas in the "late" disease, 39 days post-immunization, they were strongly diminished, and there was an accompanying reduction in $NG2^+/O4^+$ pre-oligodendrocytes and GST-π mature oligodendrocytes. These results, together with the observed steady-state amount of $NG2^-/O4^+$ pre-myelinating oligodendrocytes, suggested that oligodendroglial precursors attempted to compensate for the progressive loss of myelin, although these cells appeared to fail to complete the last step of their differentiation program. Our findings confirm that this chronic model of EAE reproduces the features of neocortex pathology in progressive MS and suggest that, despite the proliferative response of the oligodendroglial precursors, the failure to accomplish final differentiation may be a key contributing factor to the impaired remyelination that characterizes these demyelinating conditions.

Introduction

Experimental autoimmune encephalomyelitis (EAE) is a widely used animal model characterized by inflammatory demyelination of the central nervous system (CNS), as occurs in the human disease multiple sclerosis (MS). Disease heterogeneity in terms of clinical course and neuropathology is characteristic of MS (Lucchinetti et al., 2000) and is also a feature of EAE. In fact, in the latter, depending upon the species, strain, immunization protocol and dosage of the immunogen, relapsing–remitting or chronic models can be reproduced (Berard et al., 2010; Gold et al., 2006). In patients with progressive MS the brain is globally affected, as a consequence of the persistent and diffuse inflammatory process, showing diffuse demyelination, axonal loss and microglial activation in normal appearing WM as well as in deep and cortical grey matter (GM) (Bø et al., 2003; Bø, 2009; Kidd et al., 1999; Kutzelnigg et al., 2005; Peterson et al., 2001; Rudick and Trapp, 2009). Although only a marginal correlation between focal WM lesions and cortical

Abbreviations: CNPase, 2′,3′-Cyclic Nucleotide 3′-Phosphodiesterase; dpi, days post MOG immunization; EAE, Experimental Autoimmune Encephalomyelitis; GFAP, Glial Fibrillary Acidic Protein; GRP, glial restricted progenitor; GST-π, glutathione S-transferase isoform-π; MBP, Myelin Basic Protein; MOG, Myelin Oligodendrocyte Glycoprotein; MS, Multiple Sclerosis; NeuN, Neuronal Nuclei; NF, Neurofilament; NG2, Nerve-glial antigen 2; OPC, oligodendrocyte precursor cell; PCNA, proliferative cell nuclear antigen; PDGFR-α, platelet derived growth factor receptor-α; PLP, Proteolipid Protein.

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pathology has been described, demyelination within cortical GM areas may contribute to disease progression and also play a role in the emergence of cognitive deficits (Chen et al., 2004; De Stefano et al., 2003; Geurts et al., 2009; Kutzelnigg et al., 2005; Lazeron et al., 2000; Stadelmann et al., 2008). Conventional EAE mouse models, including MOG-induced EAE, have been most commonly adopted to focus on spinal cord inflammation/demyelination (Gold et al., 2006), while neocortex demyelination has been primarily described in specifically designed experimental models (Pomeroy et al., 2005; Merkler et al., 2006; Storch et al., 2006). Data on cortical lesions were reported by Rasmussen et al. (2007) in relapsing–remitting rodent EAE induced by PLP and only very recently, forebrain demyelination has been demonstrated in chronic EAE, induced by MOG in C57BL/6 mice, that mimics primary and secondary progressive MS (Mangiardi et al., 2011).

Concurrently with destructive events, regenerative processes take place in MS and EAE demyelinated lesions of WM, including attempts by nervous tissue to remyelinate the damaged areas (Albert et al., 2007; Blakemore, 1974; Bunge et al., 1961; Franklin and ffrench-Constant, 2008; Patani et al., 2007; Prineas et al., 1993). The heterogeneity observed in the degree of remyelination in samples collected from autopsies and biopsies could be related to patients' age and MS clinical subtypes (Frohman et al., 2006; Lassmann et al., 1997; Goldschmidt et al., 2009; Patrikios et al., 2006). It has been demonstrated that some chronic MS lesions contain immature oligodendrocytes, which can be involved in the phenomenon of remyelination (Chang et al., 2000, 2002; Wolswijk, 1998, 2002), but a comprehensive analysis of the sequential maturation stages of oligodendrocyte lineage during chronic evolution of the disease is still lacking.

In the perinatal period of normal brain development, oligodendrocytes arise from precursors that differentiate through a series of stages identified by specific markers: glial restricted progenitors (GRPs) recognized by the phenotype marker A2B5 which corresponds to a specific group of gangliosides (Cameron and Rakic, 1991; Kundu et al., 1983; Liu et al., 2002; Steiner et al., 2007; Strathmann et al., 2007), oligodendrocyte precursor cells (OPCs) identified by NG2 (nerve-glial antigen 2) chondroitin sulphate proteoglycan and by PDGFR-α (platelet derived growth factor receptor-α), molecules both involved in cell proliferation and migration (He et al., 2009; Heldin and Westermark, 1999; Chekenya et al., 2008; Kucharova and Stallcup, 2010; Makagiansar et al., 2007), pre-oligodendrocytes also expressing NG2 and identified by the phenotype marker O4, which recognizes specific glycolipids and cholesterol (Bansal et al., 1989; Baumann and Pham-Dihn, 2001; Cai et al., 2006; Guardia Clausi et al., 2010; Probstmeier et al., 1999; Sommer and Schachner, 1981), myelinating oligodendrocytes that express myelin-associated proteins, MBP and MOG, and specific enzymes, CNPase and GST-π (Baumann and Pham-Dihn, 2001; Quarles, 1997; Tansey and Cammer, 1991). In the adult CNS, NG2-expressing cells, OPCs/polydendrocytes and pre-oligodendrocytes are still present. Polydendrocytes are morphologically and antigenically indistinguishable from OPCs but in normal conditions they represent a non-proliferating, stable cell population (Butt et al., 2005; Fruttiger et al., 1999; Goldman, 2005; Levine et al., 1993; Nishiyama et al., 1996, 2009).

Although several studies have described the presence of cells of the oligodendrocyte lineage in EAE demyelinating lesions of the spinal cord, relatively little is known about the situation in the cerebral cortex (Di Bello et al., 1999; Gensert and Goldman, 1997; Keirstead et al., 1998; Papadopoulos et al., 2010; Polito and Reynolds, 2005; Reynolds et al., 2002). Considering that the origin, identity and degree of maturation of the cells that may play an effective role during the remyelination process remain of primary interest, also in view of observations made on human MS lesions, in this study we have analyzed the presence and distribution of oligodendrocyte lineage cells in the cerebral cortex of MOG-induced chronic EAE. The antigenic phenotype of oligodendroglia precursors and myelinating oligodendrocytes has been revealed by a broad panel of cell-specific markers during "early" and "late" stages of the disease with the aim of defining the mode, extent, and course of the oligodendrocyte response in relation to the neocortex demyelination events.

Materials and methods

EAE induction and clinical evaluation

Procedures involving animals and their care were conducted in conformity with the institutional guidelines in compliance with national (D.L. n. 116, G.U., suppl. 40, Feb. 18, 1992) and international laws and policies (EEC Council Directive 86/609, OJ L 358, 1, Dec.12, 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996). The protocols for the proposed investigation were reviewed and approved by the Animal Care and Use Committees (IACUC) of the "Mario Negri" Institute for Pharmacological Research. Chronic EAE was induced in C57BL/6 wild type female mice (6–8 weeks of age) obtained from Harlan (Bresso, MI, Italy) and maintained in specific pathogen-free conditions. EAE was induced by subcutaneous immunization with a total of 200 μg of $MOG_{35–55}$ in incomplete Freund's adjuvant (Sigma, St. Louis, MO, USA), supplemented with 8 mg/ml of Mycobacterium tuberculosis (strain H37RA; Difco, Detroit, MI, USA). The mice received 300 ng of pertussin toxin (Sigma) i.v. at the immunization time and 48 h later. Control C57BL/6 female mice received a subcutaneous injection of incomplete Freund's adjuvant without $MOG_{35–55}$. Weight and clinical score (*cs*) were recorded daily according to the standard EAE grading scale. The onset of EAE clinical signs was at 13–14 days post-immunization (dpi). On the total immunized mice (n = 16), a first group of mice was sacrificed during "early EAE" (n = 9; *cs* 1.5 to 3.5) at 20 dpi, while a second group was followed up to 39 dpi, defined as "late EAE" (n = 6; *cs* 2.0 to 3.0). Individual clinical scores were plotted *per* day, data were expressed as median ± SEM (Supplementary Fig. 1). For each experimental group, healthy controls (n = 5) were sacrificed at equivalent times.

Histology and Immunohistochemistry

Mice were anesthetized with chloral hydrate (3 μl/g, intraperitoneal injection) and transcardially perfused with 100 ml of fixative (2% paraformaldehyde plus 0.2% glutaraldehyde). After perfusion, each hemisphere was cut into 20-μm thick sagittal sections then immunostained for light microscopy or confocal laser microscopy, except for sections that were stained with toluidine blue for comparative microanatomy analysis. The following primary antibodies were utilized in single and multiple immunolabelings: anti-MBP (Myelin Basic Protein), anti-A2B5, anti-NG2 (nerve-glial antigen 2), anti-NeuN (Neuronal Nuclei), anti-O4, anti-PDGFR-α (platelet derived growth factor receptor-α), anti-CNPase (2′,3′-Cyclic Nucleotide 3′-Phosphodiesterase), anti-MOG (Myelin Oligodendrocyte Glycoprotein, anti-GST-π (glutathione S-transferase isoform-π), anti-GFAP (Glial Fibrillary Acidic Protein), anti-NF (70 kDa Neurofilament), anti-CD45, anti-PCNA (Proliferative Cell Nuclear Antigen). The initial analysis of both cerebral cortex and subcortical white matter myelination levels, in healthy and EAE-affected mice, was carried out by immunoenzymatic methods to reveal the myelin marker MBP. Subsequently, adjacent sections were immunolabeled for laser confocal analysis with a number of markers (Table 1), according to the protocols described in the Supplementary materials. Sections were examined under a Leica TCS SP5 confocal laser scanning microscope (Leica Microsystems, Mannheim, Germany).

Quantitative assessment

Brains from healthy (n = 5), "early EAE" (20 dpi; n = 5), and "late EAE" (39 dpi; n = 5) mice were utilized for computer-aided morphometric analysis. The levels of brain myelination, in cerebral cortex